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ANNUAL PROGRESS REPORT

Grant#: N00014-91-J-1593

PRINCIPAL INVESTIGATOR: Dr. Stephen J. Benkovic

INSTITUTION: The Pennsylvania State University

GRANT TITLE: The Use of Combinatorial Heavy & Light Chain Libraries and Site Specific Mutagenesis to Create Antibody Biosensors for Metal Ions

REPORTING PERIOD: June 1, 1991 - May 31, 1992

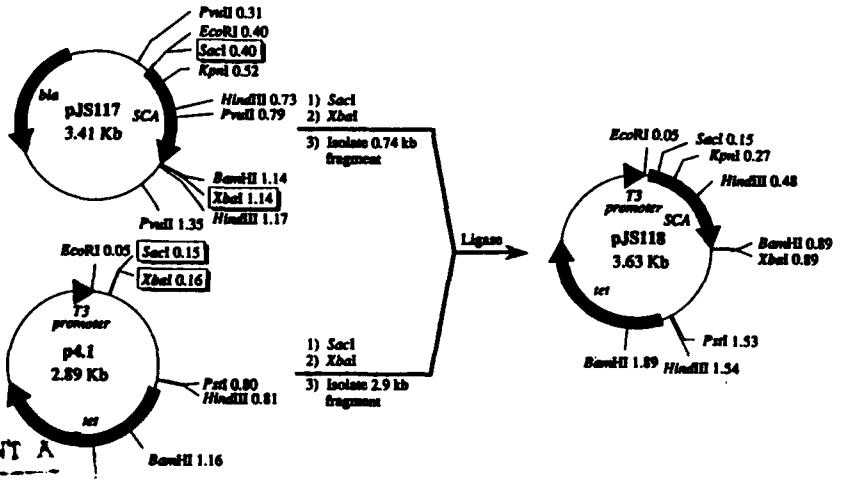
AWARD PERIOD: April 1, 1992 - March, 31, 1994

OBJECTIVE: To construct F_{ab} fragments that possess metal ion binding ligands in juxtaposition to the antigen combining site, so that the binding of both antigen and metal produces observable chemical or spectral changes in the antigen.

APPROACH: A recursive protocol will be used to create the required F_{ab} fragments. i) Antibodies with the desired binding functions will be induced with the appropriate immunogen and F_{ab}s obtained from recombinant libraries; ii) F_{ab}s will be screened for antigen binding and those with appropriate affinities isolated, sequenced and overexpressed in *E.coli*; iii) The structures of selected F_{ab}s will be deduced from modeling (in collaboration with Drs. Getzoff and Roberts, Scripps Clinic) and amino acid motifs required for metal ion binding will be introduced by site specific mutagenesis; iv) purified F_{ab}s will be tested for their metal ion affinity and response to a transducing substrate; v) improvements in the binding and catalytic properties of the F_{ab} will be sought by additional rounds of mutagenesis and/or chain shuffling with the original light or heavy chain libraries.

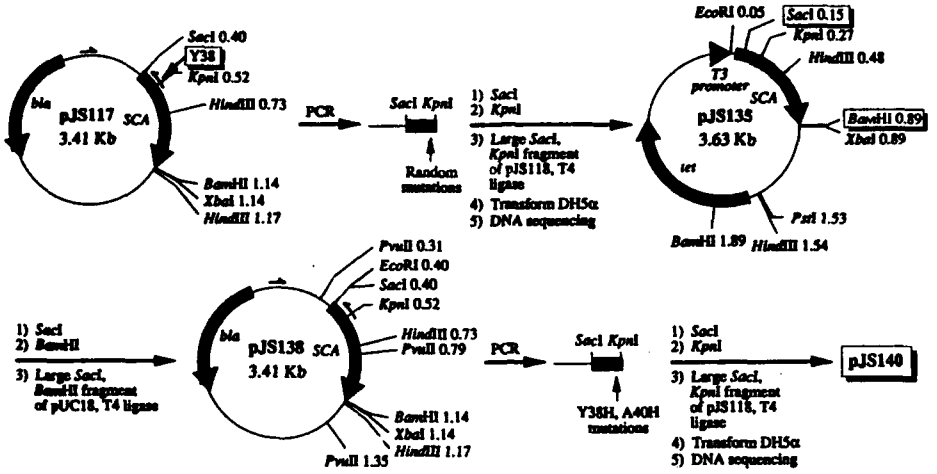
ACCOMPLISHMENTS (last 12 months): We have selected the antibody, 43C9, which efficiently catalyzes the hydrolysis of aromatic esters and anilides as a monoclonal, and have converted it to a single chain form, 43C9SCA. We have altered through subcloning the expression vector as shown in Scheme 1 to permit secretion of the SCA from *E.coli* to ease purification problems.

Scheme 1



A sequence of concentration of the supernatant after centrifugation followed by dialysis into low salt and elution from a polyCAT A column provides 0.6 of purified 43C9SCA per liter. Site specific mutagenesis has introduced Tyr38 to HIS and Ala40 to HIS mutations into the original construct, providing three histidines needed for the Zn^{2+} binding pocket in the light chain, one is already present in the wild-type SCA. Molecular modeling suggests that this

Scheme 2



placement of the metal ion should not perturb the antigen binding, but would juxtapose the metal ion and the carbonyl of the substrate.

SIGNIFICANCE: We can now express and rapidly purify the SCA at useful levels as well as execute the desired mutations.

WORK PLAN (next 12 months): The specific objective of the next year's work plan is to test the Y38H, A40H double mutant for its ability to bind metal ions, its specificity for a given metal ion, and the ability to "read out" the presence of a given metal by its affect on the spectrum of a chromophoric antigen or through its catalytic role in cleaving an ester or anilide substrate. A second site for a metal ion will also be introduced.

ANNUAL REPORT QUESTIONNAIRE
(for ONR use only)

Principal Investigator Name: Stephen J. Benkovic

Institution: The Pennsylvania State University

Project Title: The Use of Combinatorial Heavy & Light Chain Libraries and Site Specific Mutagenesis to Create Antibody Biosensors for Metal Ions

Number of ONR supported

Papers published in refereed journals: -0-

Papers or reports in non-refereed publications: -0-

Books or book chapters published: -0-

Number of ONR supported patents/inventions

Filed: -0-

Granted: -0- **Patent name and number:** -0-

Number of presentations: Total ONR Project

Invited: -0-

Contributed: -0-

Trainee Data (only for those receiving full or partial ONR support):

	TOTAL	FEMALE	MINORITY	NON-US CITIZEN
No. Grad. Students:	2	2		2
No. Postdoctorals:	1	1		1
No. Undergraduates:				

AWARDS/HONORS TO PI AND/OR TO MEMBERS OF PI'S RESEARCH GROUP (please describe):

See attached CV

Equipment purchased on grant (number and description of items costing >$1,500):

N/A